

Handwritten: F3
-cells of the immune system.

REMARKS

Applicants' attorney wishes to thank the Examiner in charge of the application for the courtesies extended to him at the interview on October 2, 2001 at which time, the last response was discussed.

The present amendment is being submitted in place of the previous amendment so as to put everything in one amendment. The specification has now been amended to insert reference to the PCT application and to correct a formula on page 12. In addition, claims 20 to 44 have been cancelled and have been replaced by present claims 60 to 74. Claims 45 to 59 are in an unentered amendment. Claim 65 recites the sequence ID numbers as requested by the Examiner at the interview. Also, Applicants are submitting herewith an amended Abstract. It is believed that the present amendment and the Abstract together put the application in condition for allowance.

Applicants request favorable reconsideration of the application in view of the amendment presented herewith.

Respectfully submitted,
Bierman, Muserlian and Lucas

By: Donald C. Lucas
Donald C. Lucas
Attorney for Applicants
Tel.# (212) 661-8000

DCL:CAM:ds
Enclosures

CERTIFICATION OF FACSIMILE TRANSMISSION

I hereby certify that this paper is being facsimile transmitted to the Patent and Trademark Office on the date shown below.

Donald C. Lucas

Donald C. Lucas

October 11, 2001

ABSTRACT OF THE DISCLOSURE

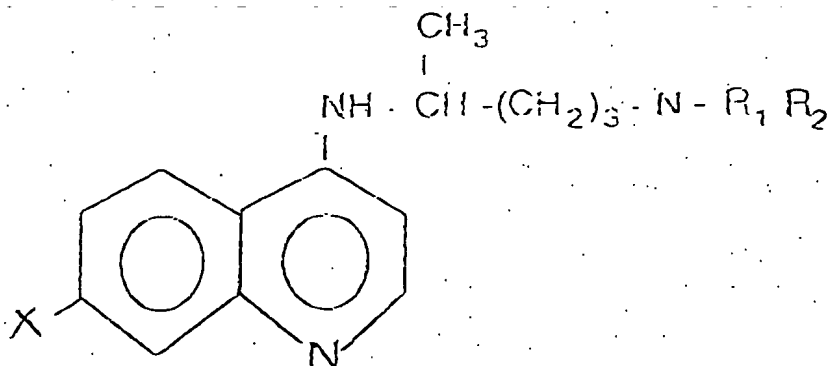
The complex has at least one negatively charged nucleic acid bonded to at least one positively charged polymeric conjugate.

The conjugate containing a polylysine formed from monomers having free NH_3^+ groups, and having at least 10% of the free NH_3^+ groups substituted by residues which can be protonated in a weakly acid medium causing destabilization of cell membranes.

Optionally, some of the free NH_3^+ groups can be substituted by a molecule with a recognition signal by a cell membrane receptor.

F4 The free NH_3^+ groups of the said polylysine make up at least 30% of the monomers of the polymeric conjugate.

The residue that causes the destabilization of cell membrane in weak acid of quinolines of the formula:



where R₁ is hydrogen, R₂ is $-(\text{CH}_2)_n-\text{CO}_2-\text{H}$, X is hydrogen or chlorine and n is an integer from 1 to 10.

The signal is a simple oside or a disaccharide or peptide.

WO 98/22610

PAGE 1 OF SPEC.

PCT/FR97/02022

--This application is a 371 of PCT/FR97/02022 filed November 10, 1997.--

NOVEL POLYMERIC COMPLEXES FOR THE TRANSFECTION OF NUCLEIC ACIDS,
WITH RESIDUES CAUSING THE DESTABILISATION OF CELL MEMBRANES.

The invention relates to new complexes of nucleic acids and polymer substituted by
5 residues which cause destabilization of cell membranes.

The introduction of a foreign gene into a cell is the basis of genetic treatment. The
transfer of genes can be achieved using either a modified viral material (vaccine virus,
retrovirus, adenovirus or herpes virus) or using non-viral vectors (cationic lipids, liposomes).
The former, although effective, have safety problems. As regards the latter, the effectiveness
10 is greatly reduced in the presence of serum, and as a result their use is restricted to *in vitro* or
ex vivo.

Polylysine, which can form stable electrostatic complexes with a plasmid DNA is the
basis for development of non-viral vectors for transfer of genes in animal cells.

Complexes of DNA and unsubstituted polylysine generally are not effective for
15 transfection of cells because of the very high stability of the complexes (and therefore weak
dissociation and salting out of the DNA) under physiological conditions as a consequence of
a very high co-operativity of polycation-polyanion interactions.

The transfection efficiency can be improved if the number of charges present on the
polypeptide is decreased in order to reduce the interactive forces between the DNA and the
20 polylysine. For example, if 40% of the ϵ -NH₂ functions of the lysine residues of the
polylysine are partly neutralized by polyhydroxyalkanoyl derivatives, such as δ -
gluconolactone, the DNA/partly gluconylated polylysine complexes are more effective than
DNA/polylysine complexes in transfection of cells.

The polylysine can be substituted by specific receptor ligands which are present on
25 the surface of cells and are capable of inducing specific endocytosis of complexes with a
plasmid DNA by target cells.

Conjugates obtained by substituting polylysine by asialoorosomucoid, transferrin,
insulin, immunoglobulin and growth factors have been proposed as plasmid guide vectors.
However, these protein ligands render the complexes highly immunogenic.

30 The polylysine can be substituted by low molecular weight ligands which are less
immunogenic than the osides and oligosides recognized by specific membrane receptors
(membrane lectins) on the surface of target cells. Glycosylated polylysine has been proposed

F

WO 98/22610

PAGE 12 OF SPEC.

PCT/FR97/02022

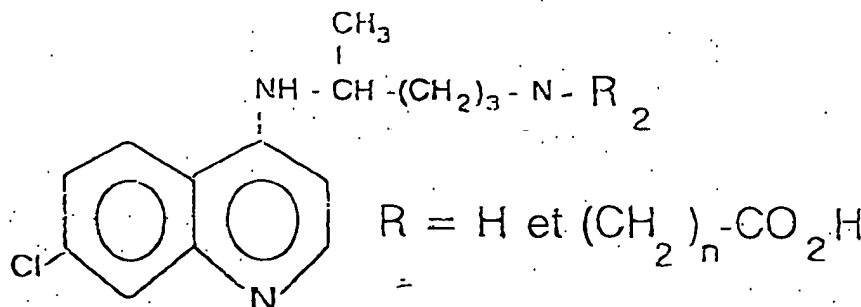
12

substituted polymer, that is to say 1 molecule for about 50 molecules of the substituted polymer.

- for a signal molecule of high affinity with respect to its receptor (K_a between 10^5 l/mole and 10^7 l/mole), about 0.5 to about 10, advantageously 1 molecule for about 200 monomer units of the substituted polymer, that is to say 1 molecule for about 1 molecule of substituted polymer;

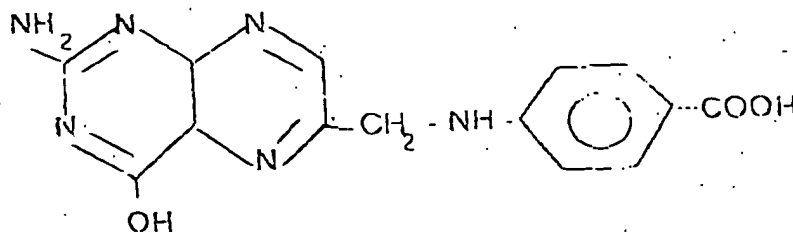
- for a signal molecule of moderate affinity with respect to its receptor ($K_a < 10^5$ l/mole), about 10 to about 100, advantageously 50 molecules for about 200 monomer units of the substituted polymer, that is to say 50 molecules for about 1 molecule of substituted polymer.

The family of quinolines is represented by the following formula:



in which n has a value from 1 to 10, preferably 1 to 3.

The family of pterines is represented by the following formula:



MARKED UP VERSION OF CLAIMS

410.015

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: : D. Nguyen
MIDOUX et al :
Serial No.: 297,519 : Group: 1633
Filed: May 3, 1999 :
For: NOVEL POLYMERIC... MEMBRANES :

600 Third Avenue
New York N.Y. 10016
September 19, 2001

AMENDMENT

Asst. Commissioner for Patents
Washington, D.C. 20231

Sir:

Responsive to the office action of July 31, 2001, please amend
this application as follows:

IN THE CLAIMS:

Claim 45 (amended) A complex comprised of at least one
negatively charged nucleic acid and at least one positively charged
polymeric conjugate with the bond therebetween being electrostatic
in nature,

the polymeric conjugate containing a polylysine formed from
monomers having free NH_3^+ groups,

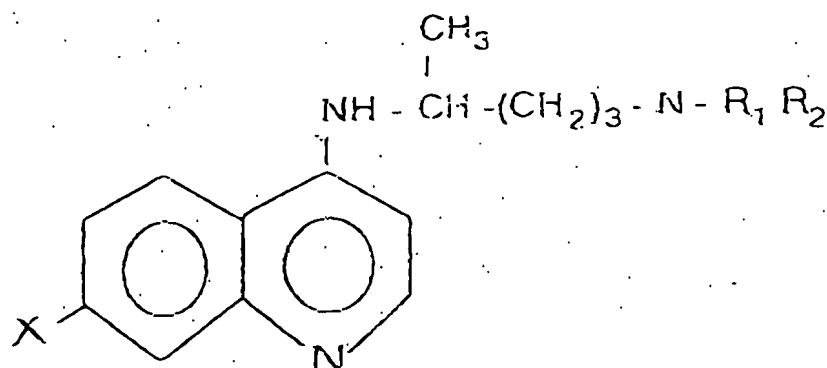
at least 10% of free NH_3^+ groups of the said polylysine are
substituted by residues which can be protonated in a weakly acid
medium causing destabilization of cell membranes,

and optionally at least one free NH_3^+ group of the said
polylysine is substituted by a molecule with a recognition signal
recognized by a cell membrane receptor,

F

with the proviso that all the free NH_3^+ groups of the said polylysine make up at least 30% of the number of monomers of the skeleton of the polymeric conjugate,

wherein said residues causing destabilization of cell membrane in a weakly acid medium belong to the family of quinolines of the formula:



in which R_1 is hydrogen, R_2 is $-(\text{CH}_2)_n-\text{CO}_2-\text{H}$, X is hydrogen or chlorine and n is an integer from 1 to 10, wherein said recognition signal is selected from the group consisting of:

a) simple osides selected from the group consisting of α or β conformers of 2-deoxy, 2-amino or 2-deoxy, 2-acetamido neutral monosaccharides; α or β conformers of glycuronic acid derivatives of neutral monosaccharides; α or β conformers of L-iduronic acid, of keto-deoxy-octonic acid, of N-acetyl neuraminic acid, or of N-glycoloyl-neuraminic acid; and α or β conformers of neutral 6-deoxy monosaccharides;

b) a disaccharide selected from the group consisting of lactose and mannopyranosyl α -6-mannopyranose,

c) complex osides selected from the group consisting of Lewis^a, ...

Lewis^b, Lewis^a, oligomannosides and oligolactosamines and
d) peptides.

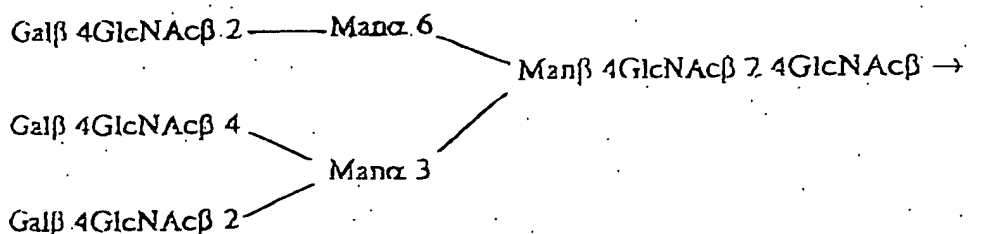
Claim 46 (amended) The complex of claim 44 wherein said
quinolines are selected from the group consisting of 7-chloro-4-
(amino-1-methylbutylamino)-quinoline, N⁴-(7-chloro-4-quinolinyl)-
1,4-pentanediamine, 8-(4-amino-1-methylbutylamino)-6-
methoxyquinoline (pyrimaquine), N⁴-(6-methoxy-8-quinolinyl)-1,4-
pentanediamine, and pyridines selected from the group consisting of
nicotinic acid and quinolenic acid and pterines.

Claim 47 (amended) The complex of claim 45 wherein the free
NH₃⁺ groups of the polylysine are substituted with a non-charged
gluconyl residue causing a reduction in the positive charge of the
polymeric conjugate which facilitates salting out of the nucleic
acids upon dissociation of the complex.

Claim 49 (amended) The complex of claim 45 wherein:

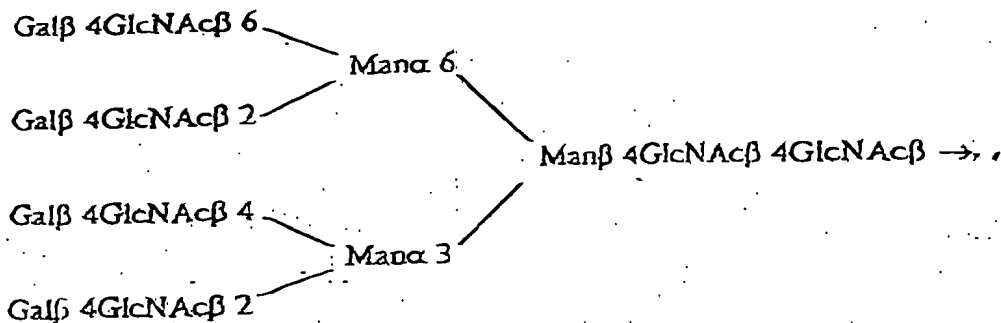
- the monosaccharide is selected from the group consisting of
galactose, mannose, fucose, glucose, ribose, xylose and rhamnose
and

- the oligosaccharide is selected from the group consisting of
(a) Asialo-oligoside of the type of triantennar lactosamine of

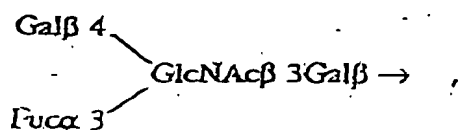


F

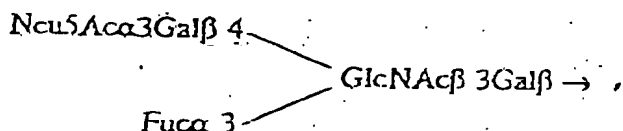
(b) Asialo-oligoside of the type of tetraantennar lactosamin
of the formula



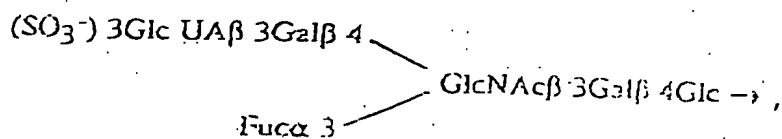
(c) Lewis x of the formula



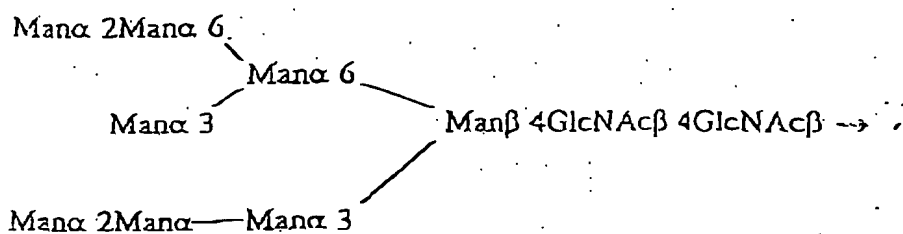
(d) Lewis x sialyl of the formula



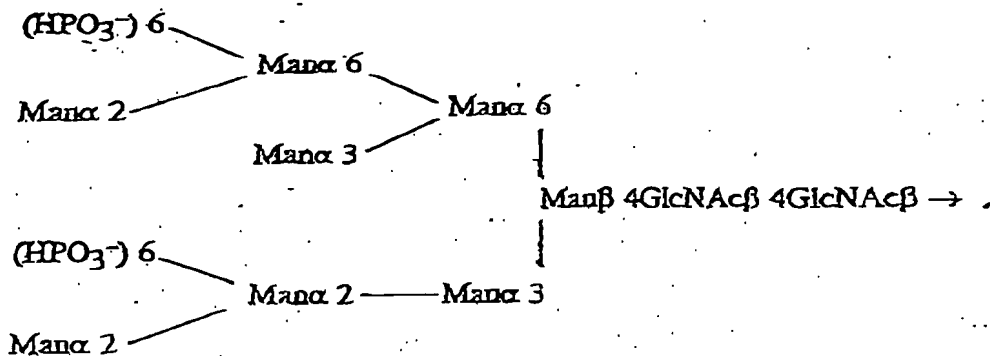
(e) Sulphated Lewis x derivative (HNK1) of the formula



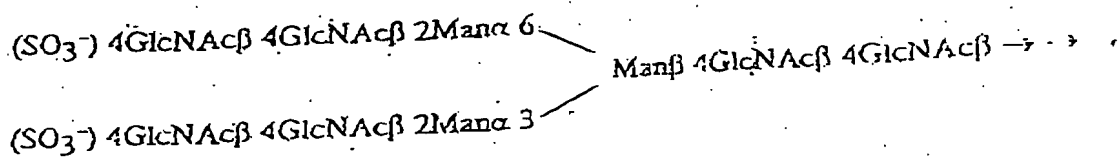
(f) Oligomannoside of the formula



(g) Phosphorylated oligomannoside



(h) Oligosaccharide of the type of sulphated lactosamine of the formula



- i. Lactose,
- j. $\text{Fuc}\alpha 2\text{Gak}\beta 3 (\text{fuc}\alpha 4) \text{GlcNAc}\beta 1\text{Gal}\beta 3\text{Glc}$,
- k. $\text{Fuc}\alpha 4 (\text{Ga}\beta 3) \text{GlcNAc}\beta 3\text{Gal}\beta$ and
- l. $\text{Man}\alpha 6\text{-man}$.

Claim 50 (amended). The complex of claim 49 wherein the peptides are selected from the group consisting of

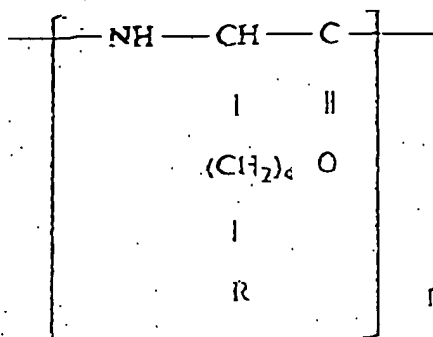
- vasodilator intestinal polypeptide (VIP)
HSDAVFTDNYTRLRKQMAVKKYLNLSILN-NH₂ (SER ID NO: 2)
- atrial natriuretic polypeptide (ANP)
SLRRSSCFGGRMDRIGAQSGLGCNSFRY (SER ID NO: 3)
- lipocortin
HDMNKVLDL (SER ID NO: 4)

- bradykinin

RPPGFSPER (SEQ ID No. 5)

peptides of intergrins, peptide hormones and chemotactics factors.

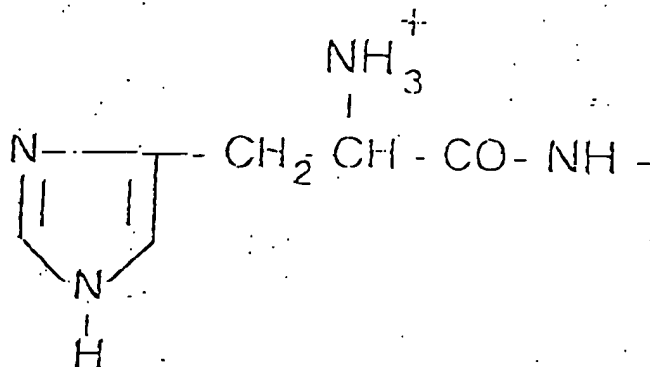
Claim 53 (amended) The complex of claim 51 wherein the polymeric conjugate has the following formula:



wherein:

- p is an integer from 15 to 900,

- 10% to 45% of R is a residue having an imidazole nucleus and optionally a free NH_3^+ , R has the formula:



or

- 30% to 90% of the number of R, having free NH_3^+ , and 0 to 45% of R are substituted by a molecule which constitutes a recognition signal by a cell membrane receptor,

with the proviso that all the free NH_3^+ functions make up at least 30% of the number of monomer units of the polymeric skeleton of the above mentioned polymeric conjugate.

Claim 56 (amended) The positively charged polymeric conjugate according to claim 55 wherein the free NH_3^+ groups of the polylysine are substituted with a non-charged residue causing a reduction in the positive charge of the polymeric conjugate which facilitates salting out of the nucleic acids upon dissociation of the complex, said non-charged residue being a gluconyl.

Claim 57 (amended) The composition comprising the complex of claim 45 and an inert pharmaceutical carrier.

Claim 58 (amended) A method of transfecting cultured cells comprising incubating said cells in the presence of a composition of claim 57 under conditions wherein said composition enters said cells, and the nucleic acid comprised in the complex of said composition is released to transfect culture cells.

Claim 59 (amended) The method of claim 58 wherein the cells are selected from the group consisting of

- cells of haematopoietic strains;
- dendritic cells;
- liver cells;
- skeletal muscle cells;
- skin cells;
- fibroblasts,
- keratinocytes,
- dendritic cells,

F

- melanocytes;
- cells of the vascular walls;
endothelial;
smooth muscle;
- epithelial cells of the respiratory tract;
- cells of the central nervous system;
- cancerous cells; and
- cells of the immune system.

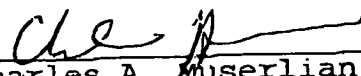
2
ad 15

REMARKS

Reconsideration of this application is requested in view of the amendments to the claims which are believed to conform to the suggestions made by the Examiner in the advisory action for which Applicants are grateful. It was indicated that the claims once corrected, would overcome the outstanding rejections. Therefore, it is believed that the application is now in condition for acceptance and favorable reconsideration of the application is requested.

Respectfully submitted,
Bierman, Muserlian and Lucas

By:


Charles A. Muserlian #19,683
Attorney for Applicants
Tel.# (212) 661-8000

CAM:ds
Enclosures